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ELECTRICAL ACTIVITY AND CELL DENSITY IN THE CLAUSTRO-INSULAR AREA OF THE RABBIT

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I. INTRODUCTION

The purpose of the prensent investigation was to study the relation between the cell density and the spontaneous spike and slow wave activity in different cortical layers. Previous investigations on the relationship between these two different types of activity and their correlation to different cortical structures have been limited to a few selected cortical layers. The aim of the present investigations was to study the distribution of spike and slow wave activities in different layers throughout the entire cortical depth. The claustro-insular area *i.e. area insularis agranularis anterior dorsalis* — Ai_1 (according to the terminology of ROSE 1928) in the rabbit has been chosen as the object of this study, since its different cortical layers with varying cell density are relatively well differentiated. The fact that this area including the claustral layer, is thicker than other parts of the cerebral cortex in the rabbit offers the possibility of recording activity from several separate points at different depths, a circumstance of significance for the statistical treatment of the material.

The spontaneous activity at different cortical depths has been recorded with an extracellular microelectrode, and the distribution of the spike potentials and the slow wave activity has been studied in different cortical layers, of which the relative cell density has been estimated on the basis of histological studies. The influence of varying depths of anesthesia on the spatial distribution of the different types of spontaneous activity has also been studied. The material obtained has been treated statistically.

Some of the results have been described in a preliminary report (VALLEALA 1960).

II. SURVEY OF MICROELECTRODE STUDIES, WITH SPECIAL REFERENCE TO THE SPONTANEOUS CORTICAL ACTIVITY

RENSHAW, FORBES and MORISON (1940) in their classical microelectrode study on the electrical activity of isocortex and hippocampus discussed the spatial accuracy of the extracellular microelectrode technique. In the hippocampus they recorded low amplitude negative spike potentials, which they assumed to represent activity in single neurons or small, functionally homogeneous neuron groups. The origin of these spike potentials, with a duration of about 1 msec, was assumed to be the perikaryon-rich layer of the pyramidal cells in the Ammon's horn or the vicinity of this layer.

WOLDRING and DIRKEN (1950) studied the spontaneous cortical activity in anesthetized rabbits. According to them the activity consisted of three components: 1) a heterogeneous background activity, 2) slow waves which were generally irregular in shape and mode of occurence and which on rare occasions appeared in spindle-shaped trains, 3) spike activity which was affected by anesthetics. Curare had no effect on the spike activity provided that ventilation was insured.

Successful attempts later on were made to refine and standardize the microelectrode method by using glass micropipettes filled with 3-M KCl solution (LING and GERARD 1949). The tips of the micropipettes are less than 1 μ in diameter and the resistance generally varies between 1 and 50 megohms. Most publications deal with the response to electrical and different types of natural stimulation but they also present information on the nature of spontaneous activity. (BAUMGARTEN & JUNG 1952; AMASSIAN 1953; BUSER & ALBE-FESSARD 1953; TASAKI *et al.* 1954; LI & JASPER 1953; PHILLIPS 1956 a; MOUNTCASTLE *et al.* 1957). All workers report the appearance of membrane potential changes when the electrode passes through the cortex. Some investigators have mainly been interested in these membrane potentials (LI 1955, 1959; PHILLIPS 1956 a and b).

Even a small electrode obviously causes damage to the cell, and the damage is naturally more severe the larger the diameter

of the electrode tip in relation to the size of the cell (see e.g. HAAPANEN *et al.* 1958). When the tip of the electrode penetrates the cell structure, so called injury potentials may appear. Before the appearance of injury potentials, *i.e.* when the electrode is being pushed towards the cell, spike potentials are often recorded within a limited zone. The polarity and configuration of these spike potentials change essentially before the microelectrode has achieved a localization at which it evokes injury potentials. These spike potentials may be called *proximity potentials.* LI (1955) has described the general features of these proximity potentials and their fluctuations as a function of the position of the electrode tip in relation to the cell. When the electrode enters the "active zone", negative potentials are recorded which may be followed by a small positive potential. When the electrode is moved further a sudden change of polarity often occurs and the potentials become positive-negative. The initial positive component is larger and grows further when the electrode is moved closer to the cell. At a certain point positive spikes of several tenths of a millivolt occur. When a momentary negative DC shift appears these positive spikes may develop into high-frequency injury potentials, which then cease abruptly.

MOUNTCASTLE *et al.* (1957) studied the depth dimension of the area from which proximity potentials could be obtained when evoked by different types of stimulation. In their studies made with slightly larger microelectrodes (tip diameter 2—4 μ), the proximity potentials were seldom found to be positive (for dimension see FRANK 1955). The only change which they obtained when the electrode was moved towards the cell was a diminution of the amplitude of negative spike potentials. Generally, injury potentials were not obtained, which led these workers to assume that in their experiments the electrode often by-passed the neuron. The depth dimension of the area from which proximity potential were obtained was found to be about 100 μ.

LI and JASPER (1953) reported that the size of the spike potentials — in their investigations negative — is affected not only by "proximity" to but also by the volume of the perikaryon of the active cell in the vicinity of the electrode tip. Thus, they recorded their maximum spike potentials from the layer of the large pyramidal cells.

These workers further stated that the zone of the proximity potentials is not markedly greater than the size of the neurosome. They assumed that the fall in the gradient of the electric field is fairly sharp and that it is difficult to map the limit of this field with high-resistance electrodes because of the high noise level. They also emphasized the difficulty in recording potentials from small cells and fibres with an electrode with such properties. The records obtained will thus represent a selected group of the spontaneously active cells.

TOWE and AMASSIAN (1958) reported that when a large spike amplitude was recorded, the area from which proximity potentials could be obtained is also large and concluded that a large volume of the nerve element is involved.

The potential fluctuations of the electrocorticogram recorded with gross electrodes, are of long time duration. The possible counterpart to the EEG slow activity in the intracortical microelectrode records has consequently attracted great attention. According to RENSHAW *et al.* (1940) it is not probable that the slow waves of the EEG are composed of spike potentials. Most workers refer to the long time factor of the dendritic potentials (CHANG 1951, 1952, 1955) which according to CLARE and BISHOP are graded (1955 a and b).

Distinct differences have been stressed between the axone and the soma response, on one hand, and the dendrite response on the other. TASAKI *et al.* (1954) for instance, stated the potential duration in the axon to be somewhat below 1 msec., in the neuronal soma slightly over 1 msec. and in the dendrites 15—20 msec.

RENSHAW *et al.* (1940) using a pair of electrodes with an interelectrode distance of 105—300 μ were able to record potentials of 30—150 msec. duration, which showed a tendency to be localised to certain cortical depths. MOUNTCASTLE *et al.* (1957) recorded a slow wave type, the spatial extent of which varied between 20 μ and 70 μ (average 32 μ). This type of activity was found in all layers of the cortex. Their preparation was under a light Penthotal anesthesia.

The relationship between spike activity and slow activity has been the object of many studies. LI and JASPER (1953) paid

attention to the more differentiated picture of the cortical activity obtained by an intracortical microelectrode than that obtained by surface electrode recording. A considerable advantage of the microelectrode technique is that an electrode of this type makes close contact with the structural elements. The spontaneous electrical activity recorded with a microelectrode can be considered as a wave spectrum in which the duration of the individual waves gradually changes from short to long. In the short duration periphery of the spectrum it is possible to distinguish the spike potentials, which are fairly sharply divided from the other part of the spectrum.

According to the above-mentioned workers these types of activity can be characterised as follows:

The *spike potentials* follow the "all-or-none" law and show a precise spatial localisation in zones of a few microns to tens of microns in diameter in the depth dimension of the cortex. The rise and fall of spike potential frequency may be regarded as signs of increase and decrease in excitability. The spike activity disappears in anoxia and in deep anesthesia.

The *slow potential* activity is usually more heterogeneous and not as well defined as the spike activity. The slow potentials are graded and not of "all or none" type. Their spatial distribution in the cerebral cortex is not as distinct as that of the spike potentials and their pattern often shows considerable variations in recordings from a single point. The variation often increases with the duration of the waves. In deep anesthesia and anoxia there is a lowering of the frequency and decrease in amplitude.

Li & Jasper (1953) considered the relationship between spike activity and slow activity to be complex and variable. According to them some units tend to discharge with slow waves; others do not. Those units which discharged with waves had a tendency to fire in bursts. Verzeano & Calma (1954) reported that the relationship between the spikes and the slow waves was completely fortuitous in the alert animal, but that these types of activity were synchronized when the animal was under deep Nembutal anesthesia characterized by spindle activity. These investigators used steel electrodes with a tip diameter ranging from 5 to 10 μ and recorded negative spikes with low

amplitude. According to them a train of negative spikes was always followed by the positive slow wave. The investigators concluded that the slow positive wave represents summated positive after-potentials. In a later investigation (VERZEANO *et al.* 1955) the various results concerning the ratio between slow activity and spike activity obtained by different workers were discussed. The divergences were attributed, at least in part, to differences in the preparation and in the recording technique. As mentioned above, MOUNTCASTLE *et al.* (1957) described a type of spatial grouped spontaneous slow wave activity which, however, was not accompanied by spike activity. It is to be noted that reports of concurrent spike activity and slow activity generally refer to spike potentials of negative polarity and relatively small amplitude.

III. SURVEY OF ANATOMICAL STUDIES OF THE CLAUSTRO-INSULAR AREA

A. General

In the present investigation the term "claustro-insular area" refers to the claustrum and the insular section of the cerebral cortex covering this nucleus. In man and primates the ventral part of the claustrum — fragmented into individual nuclei — partly extends into the temporal lobe, *i.e.* outside the traditional insula. BROCKHAUS (1940) used the concept claustro-cortex to denote the part of the cerebral cortex which covers the claustrum.

The claustro-insular area constitutes a regional entity the ontogenetic and histological homology of which has been the subject of considerable controversy. The greatest divergence of opinion has concerned the question of whether the claustrum originates as a fragment of the deepest layer of the cerebral cortex enclosing it, the insular area, or whether its genesis is independent of the cerebral cortex. The former view was upheld by *e.g.* BRODMAN (1909), ARIENS-KAPPERS (1921), ROSE (1928, 1931), and the latter by *e.g.* CAJAL (1902), VON LANDAU (1923), VON ECONOMO and KOSKINAS (1925), BROCKHAUS (1940), FILIMONOFF (1947), MACCHI (1951).

The claustrum, which lies latero-basally, is separated from the medially situated putamen by the external capsule. Laterally, the claustrum is bounded by the extreme capsule which varies greatly in size and compactness in different species. According to RAE (1954), who reviewed the literature on the claustrum and its connections, silverimpregnated sections show an intricate fibrocellular reticulum. The cell bodies are scattered without apparent order in the mesh of this network. There is no precise information concerning the possible synapses between the reticulum and the claustral cells.

ROSE (1928) described the cytoarchitecture of the insula in many animals and man. ROSE, like BROADMAN, includes claustrum in the insula. Following this line of approach, he included in the insula the pre-piriform area belonging to the claustro-cortex. The pre-piriform area is well developed in macrosmatic mammals. It is structurally related to the rhinencephalon and separated from the neo-palliar part of the insula by the rhinal sulcus. According to BROADMAN (1909) the appearance of the insula in mammals is constant and in area the largest uniform part of the cerebral cortex. It is best developed in man, but is, however, overshadowed by the other neopalliar structures.

B. The claustro-insular area in the rabbit

ROSE's description (1928, 1931) and terminology of the claustro-insular area has been followed in this paper. The insular area, which according to ROSE is agranular in lower mammals, is clearly divided in the rabbit into a dorsal granular and ventral agranular part bordering on the rhinal sulcus. The extent of the insular area is easy to estimate owing to the poor folding of the cerebral cortex of the rabbit. BROADMAN (1909) stated the ventral width of the insular area to be a third of the height of the hemisphere and its length to be more than half of the hemisphere. In the rabbit the claustrum is topographically closely connected with the cerebral cortex covering it since the extreme capsule is only partly visible but is distinguishable by the special character of its structural elements.

Fig. 1. Lateral view of the rabbit's brain. B. = Bulbus olfactorius. Rh = rhinal sulcus. The dark area above the rhinal sulcus indicates the position of Area insularis agranularis (after Rose, 1928).

The present investigation concerns the agranular part of the insular area only *(Area insularis agranularis anterior dorsalis)* (Fig. 1). The characteristics of the layers of the insular area in the rabbit can be described os follows (Fig. 2).

The zonal lamina (I) is fairly broad. Layers II and III are contiguous, containing small or medium-sized pyramidal cells which are often grouped in dense clusters. The layer corresponding to the granular layer (IV) is absent. The ganglionic cell layer (V) is broad and divided into three sublayers: a, b and c. Va and Vc are visible as rather light zones in a Nissl-stained preparation. Vb is broad and contains large, radially arranged pyramidal cells. The pyramidal cells of the underlying multiform layer (VI) are small and narrow. The claustrum is very closely connected with the sixth layer (with which layer VII is also frequently assimilated). The cells of the claustrum are of medium size and large, stellate and fusiform. In some places cells resembling the pyramidal cells are seen.

According to Zunino (1909) and Winkler & Potter (1911) the fiber structure of the insular cortex is very scanty in the rabbit. Rose (1928, 1931) reported that the rostral part of the insular area shows no fibres of the extreme capsule. Le Gros Clark Meyer (1947) by removing the olfactory bulb and tracing degenerated fibres, showed that the olfactory tract also terminates

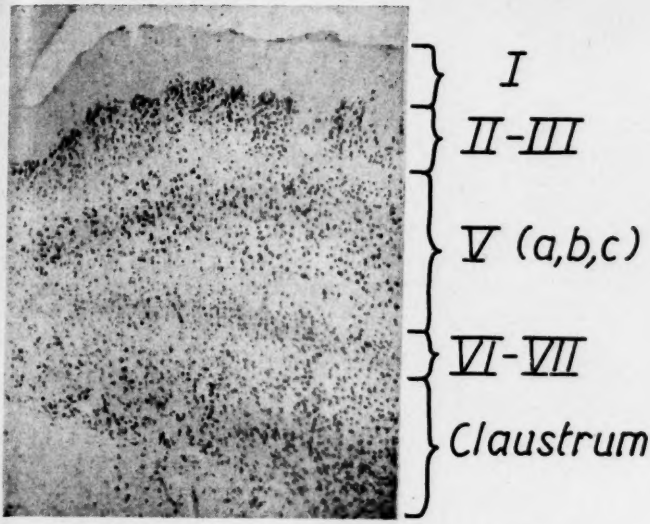

Fig. 2. Microphotograph of a transverse section through *Area insularis agranuluris* showing the different horizontal layers (nomenclature after Rose). Thionine. x 24.

in the pre-piriform area which could be regarded as an olfacto-projective area. ALLISON (1950), using a corresponding method, showed a connection between the pre-piriform area and the agranular insular area. This finding in the rabbit in a way supported the general hypothesis put forward by ROSE (1928) that the agranular insular area is closely connected with the rhinencephalon and that it participates in the olfactory function. No direct connections have been found between the area insularis anterior dorsalis (ai_1) and the hypothalamus (LUNDBERG 1960).

IV. METHODS

Animal preparation. The investigations were carried out on rabbits having a mean weight of 2.6 kg (range 2.2—3.5 kg). Before operation 30 mg/kg of Nembutal was given intravenously

and Lidocain (1 % solution) was injected around the operative field. Tracheotomy was followed by evisceration of the right eye. The orbital skull bone was removed on the right side and the anterior part of the rhinal sulcus was exposed. Bleeding was carefully stopped by ligation or cauterisation.

Approximately 5 × 5 mm of the dura was removed in the immediate vicinity of the rhinal sulcus, 7 mm occipitally from the frontal end of the fissure. A small celluloid plate with a hole (1 × 2 mm) in its centre was placed on the exposed part of the brain with the hole over the place where the dura was removed. The arterial pulsations did not seriously interfere with the recordings. The movements of the brain caused by respiration, however, was considerable at times. These movements were damped by placing a thin layer of cotton wool between the celluloid plate and the edges of the bone. Microscopic control was made in order to check that the pial circulation was intact.

The head of the animal was rigidly fixed in a holder. The holder was fitted with a ball-and-socket joint by which the head could be tilted laterally so that the surface of the brain under examination lay as nearly horizontal as possible. This permitted the electrode to be manipulated in a vertical direction in order to be inserted perpendicularly to the brain surface. The operative field was covered with paraffin kept at a temperature of ca. 38° C.

Recording. Glass capillaries filled with 2.7 M KCl were used as recording electrodes (resistance 1—20 megohms). The microelectrode was inserted vertically into the brain through the hole in the celluloid window with the aid of a Zeiss micromanipulator. The insertion was carried out under visual control through a stereomicroscope. The indifferent lead was a gross Ag-AgCl electrode placed in the subcutaneous tissue.

The potentials were recorded with the aid of a cathode follower (Haapanen & Ottoson 1954) and an AC-coupled (time constant 200 msec.) Nagard oscilloscope-amplifier. A 2-channel analyzer and a loudspeaker were connected. The time integral of the slow potentials was recorded by one channel with a coupling time constant of 500 msec. The other channel gave a count of all positive spikes exceeding 1 mV. In all experiments the ampli-

fication was kept constant at 1 mV/inch. The noise level varied 100-200 microvolts. Some potentials were photographed with a Grass camera.

The electrode was inserted into the claustro-insular area 1 mm dorsally from the rhinal sulcus and about 7 mm occipitally from the frontal end of the rhinal sulcus. This area corresponds to that described by ROSE at the anterior dorsal agranular insular area (Fig. 1 and 2). The microelectrode was inserted in steps of 50 μ and recordings were made at each 50 μ step for a period of 15 sec. If the various conditions of the preparation during the electrode insertion were found to be constant, the insertion was extended to a depth not over 3200 μ, which depth corresponds to the maximal value of the thickness of the claustro-insular area measured from histological preparations. Insertions which did not extend a depth of 2500 μ were not included in the material. The activity recorded at each step was assumed to represent the average activity at that step including a distance of 25 μ above and below the recording point.

In some cases anesthesia introduced before the operation (30 mg/kg of Nembutal intravenously) was deepened in the final stage of the operation (after about 1 ½—2 hours) with 30 mg/kg of Nembutal intraperitoneally, or 5 to 20 mg/kg of Nembutal intravenously. Lidocain was injected now and then into the skin around the wound.

The recordings were divided into two groups according to the depth of anesthesia.

The first group comprised recordings obtained at a stage in the experiment at which the animal was under light anesthesia (LA group; on an average 5 mg/kg two hours before recording). In this group curare was given as a rule in small doses (0.2—0.6 mg/kg) and the animal was maintained on artificial respiration.

The other group comprised recordings obtained under deep anesthesia (DA group; on an average 20 mg/kg two hours before and on an average 13 mg/kg one hour before recording). In order to obtain a constant level of anesthesia throughout the experiment in the DA group additional Nembutal doses (2.5—5.0 mg/kg) were given also during the recording phase (approximately every 15—30 min: c.f. FORBES *et al.* 1956). In order to avoid overdosage

the heart rate and respiration were checked regularly in the course of the experiment.

The electrode resistance was checked during the experiment. If a distinct change was noted, the recordings were rejected. The recordings were also rejected when the preparation showed signs of impaired circulation in the pial vessels or if abnormal cortical activity appeared (*e.g.* spreading depression, LEAO 1944 a and b) and in the case of abrupt change in the general condition of the animal during the experiment. As a result, the number of succesful complete insertions was relatively small in relation to the great number of experiments carried out.

The insertion depths indicated by the micrometer were divided into zones, 100 μ in width. In order to allow the recording point to represent the middle of each zone, a depth of 25 μ was taken as the upper limit of the first zone. Thus, the first zone covered 25—125 μ below the surface of the brain. In the following, 125 μ refers to the zone 25—125 μ etc. All recording data were referred to these zones. The mean of the integrator values (*i.e.* the voltage-time integrals obtained during 15 sec.) recorded from two successive electrode locations *e.g.* at the depth of 50 μ and 100 μ, was regarded to be representative for the slow activity within one zone, in this case the zone 25—125 μ (marked as 125 in the diagrams). The spike potential activity was observed on the oscilloscope and the positive spike potentials exceeding 1 mV were counted with the aid of an electronic counter. The probability for positive spike potentials to appear was calculated.

Since relatively few positive spike potentials exceeding the 1 mV amplitude level appeared, the total insertion depth was divided in larger zones, each zone including 800 μ. The number of recordings (15 sec duration) including large positive spike potentials from each 800 μ zone was expressed in per cent of all recordings made from this zone.

Distinct injury potentials were not taken into account.

In order to obtain a more detailed picture, the electrode was in some cases moved in 10 μ steps. The material so obtained was not treated statistically since the long duration of the experiment made a comparison difficult between the data obtained at the surface and those obtained from the deep layers of the cortex.

In two experiments the electrode was moved in 100 μ steps. These recordings were also excluded from the material treated statistically.

Histological preparation. The rabbit was chosen because this animal is characterized by few folds in the cerebral cortex, which helps in the assessment of the histological architecture (Bok 1929).

Histological sections were actually taken from each animal which had been used for electrophysiological analysis. However, it was found that the electrode insertions often caused structural injuries which made a systematic cell analysis difficult. Therefore, histological material was also collected from intact rabbits which had not been subjected to the electrophysiological experiment. The weights of these rabbits — 28 in all — were of the same order of magnitude as of those of the rabbits which were used in the physiological experiments, their mean weight being 2.7 kg (range 2.3—2.8 kg). The sections were taken from the region corresponding to that from which the recordings were made.

The brains were perfused via the carotid artery, first with Ringer's solution and then with 10 per cent formalin. The right hemisphere was detached after perfusion and kept in 10 per cent formalin solution for 1—2 days. The sections were cut from a block detached perpendicularly to the surface of brain. The sections, 40 μ thick, were prepared as follows: rinsing in running water, freezing and cutting: staining (0.1 per cent Thionine, distilled water, 95 per cent alcohol, absolute alcohol, xylene, Canadabalsam).

One section from each of the 28 animals was selected for the histological analysis. The perikaryon projections to be seen in the section are of course frequently smaller than the entire maximal section area of the cell. The cell projections from each section were drawn on millimeter paper with the aid of a carbon arc projector. Glial cells were not drawn. The magnification was 250 x. The tracing was made directly from a rectangular area of the same depth as the claustro-insular area and of a width of 200 μ, perpendicularly oriented to the surface of the brain and located 1 mm dorsally from the rhinal sulcus — corresponding to the area of the microelectrode insertions. The rec-

tangles were divided into 100 μ zones, beginning from a depth of 25 μ. The total number of cell projections exceeding a section surface of 80 μ^2 was counted for each zone ($100 \times 200 \times 40$ μ). Projections of cells appearing to be of borderline area (80 μ^2) were estimated by line sampling using a modification of the method reported by UOTILA and KANNAS (1952). When a calculation of this kind is used it is necessary to take into consideration also the parts of cell projections that are formed by the horizontal and vertical boundaries of the zone. These parts were excluded when they were below the lower limit.

Cells with a projection area smaller than 80 μ were not taken into consideration in the calculation in order to achieve a relevant correlation between morphological and functional data, since it was not possible with the aid of a microelectrode to record succesfully from the smallest perikarya. Satisfactory resting potentials have been successfully measured in the spinal interneurons only in cells with an estimated diameter larger than 10—20 μ (HAAPANEN *et al.* 1958). The electrodes used in the present investigation were however generally slightly larger than those used by HAAPANEN *et al.* If the area (80 μ^2) chosen as the lower limit in the present investigation is assumed to be circular, its diameter is about 10 μ. It is possible that transient injury potentials were often caused by small cells, but no distinct injury potentials were taken into account in the present investigation.

The *largest perikaryon* projections were separated from the population measured by means of the above-mentioned "line sampling" method. Projections larger than about 200 μ^2 were included in this separate group. If this area is assumed to be circular, its diameter is about 16 μ. Since the large projections were relatively few in number, a rougher zonal division was used, as in the treatment of positive spike potentials. The rectangle 200 μ in width described above was divided into zones of 800 μ. Projections larger than 200 μ^2 were counted as the per cent of all projections within the zone.

The method chosen for cytological analysis is thus concerned with two dimensional cell projections and not with cell volumes. In general terms, it can said that the larger the perikaryon the

greater is the projection. Although the size of the projection also depends on the position of the cell in three dimensional space, it gives a relative measure of the size of the neuron. The intention of this histological analysis was not to measure absolute quantities but to describe the relative amount of cells of varying magnitudes at different depths.

The line sampling was always performed in the same direction, *i.e.* parallel to the tangent to the surface of the cerebral cortex. This procedure was regarded as sufficiently accurate since pyramidal cells within the claustro-insular area are arranged uniformly with the apex of the pyramid directed radially to the surface of the brain. If this were not so, one projection of a pyramidal cell calculated as above would give a different area value from a projection of the same pyramidal cell in another position. The only exception consists of certain pyramidal cells in layers II—III of the insular area and in the claustrum proper. In the present work, however, the number of irregularly situated "pyramidal cells" was so small that the error in area determination was hardly significant.

The term "number of cells" mentioned in the treatment of the results refers to the number of perikaryon projections.

Statistical methods. The statistical treatment of the material comprises 32 zones. These zones represent "individuals" whose morphological and physiological characteristics are compared. The mutual independence of the "individuals" thus selected is fairly great owing to the special nature of the recording technique employed. The microelectrode is capable of selectively recording the activity from a small tissue volume. Thanks to the recording technique, each zone exhibited electric properties which made it possible to distinguish the zones. The possibility of treating the statistical qualities of the electrophysiological material within the above zonal distribution is also based on this "individuality" of the zones. This would not be possible, if larger electrodes had been used.

Standard methods of statistical analysis were applied to the data thus obtained, using the following formulae.

If the arithmetic mean of a variable x, which represents a certain characteristic in the sample of n individuals, is denoted

by $\bar{x}$, the standard deviation of x, viz. s_x, was obtained from the

$$s_x^2 = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2$$

and the standard error of the mean from the formula

$$s\{\bar{x}\} = \frac{s_x}{\sqrt{n}}.$$

To estimate the level of significance of the difference of two means, $\bar{x}$ and $\bar{y}$, the following calculation was made:

$$t = \frac{\bar{x} - \bar{y}}{s\sqrt{\frac{1}{n_x} + \frac{1}{n_y}}},$$

in which

$$s^2 = \frac{(n_x - 1)\, s_x^2 + (n_y - 1)\, s_y^2}{n_x + n_y - 2}$$

and the distribution of which is Student's distribution with the following number of degrees of freedom: $f = n_x + n_y - 2$.

The standard error of the percentage p was calculated from the formula

$$s\{p\} = \sqrt{\frac{p(100 - p)}{n}}.$$

The significance of the difference between two percentages was also estimated with the t-test using the following calculation

$$t = \frac{p_1 - p_2}{s\{p_1 - p_2\}},$$

where

$$s^2\{p_1 - p_2\} = s^2\{p_1\} + s^2\{p_2\}$$

and the distribution of which is the Student distribution with the following number of degrees of freedom

$$f = n_1 + n_2 - 2.$$

The correlation coefficient between two variables x and y was calculated from the formula

$$r = \frac{s_{xy}}{s_x s_y},$$

where

$$s_{xy} = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})$$

and s_x and s_y represent the standard deviations of x and y, respectively.

To find out at which level of significance the correlation between x and y differs from the 0, the t-test was again used, using the formula

$$t = \frac{r}{\sqrt{1 - r^2}} \cdot \sqrt{n - 2},$$

number of degrees of freedom $f = n - 2$.

The coefficient of partial correlation between variables 1 and 2 taking variable 3 as constant, was calculated from the formula

$$r_{12 \cdot 3} = \frac{r_{12} - r_{13}\, r_{23}}{\sqrt{(1 - r_{13}^2)(1 - r_{23}^2)}}.$$

When drawing conclusions with the aid of the t values, the risk P was obtained from statistical tables. P indicates the level of significance, *i.e.*, the probability that the difference observed is due to chance. As has been often customary, the following expressions were used to interpret the different levels of significance:

Not significant (—)	$0.05 < P$
Significant (s.)	$0.01 < P \leq 0.05$
Highly significant (h.s.)	$0.001 < P \leq 0.01$
Very highly significant (v.h.s.)	$P \leq 0.001$.

V. RESULTS

A. Density of claustro-insular cells

The microphotograph of a Thionine-stained claustro-insular preparation in Fig. 2 gives a general idea of the periodic variation of cell density at different cortical depths. As mentioned above, the fluctuation of the cell density called for the designation of various horizontal layers as seen in Fig. 2.

The histological material in the present work was obtained from 28 animals, from each of which only one section was taken. These animals were not used in the physiological experiments (see above).

Although the purpose of the present work was not to localise the recording points within each "classical" layer, it was none the less desirable to obtain a general idea of the depths of the various horizontal layers in the present material. A small magnification of the histological structure of the claustro-insular area stained by the Nissl method (Fig. 2) shows four principal perikaryon layers: II—III, V b, VI—VII and the claustrum. Among these layers, VI—VII is the narrowest and least distinct.

In all preparations, the depths were measured from the surface of the cerebral cortex to the superficial boundary of the four principal perikaryon layers. The total depth of the claustro-insular area was also measured. The first boundary *i.e.* between

Table 1. Depths in μ of the borderlines between different horizontal layers in the claustro-insular area from 28 histological preparations (mean, standard deviation and standard error).

Depth / Borderline	Mean $\bar{x}$	St. D s_x	St. E $s\{\bar{x}\}$
I/II—III	320 μ	40 μ	8.2 μ
Va/Vb	810 μ	100 μ	18.9 μ
Vc/VI—VII	1710 μ	200 μ	38.2 μ
VI—VII/claustrum	2000 μ	250 μ	47.3 μ
claustrum/white matter	2740 μ	350 μ	66.5 μ

Table 2. Number of cells (column 2) per unit zone (see text) at different depths (mean, standard deviation and standard error). 28 histological preparations from the claustro-insular area.

Zone (μ)	Mean $\bar{x}$	St. D s_x	St. E $s\left\{\bar{x}\right\}$
125	—	—	—
225	—	—	—
325	1.5	2.3	0.43
425	6.9	5.8	1.1
525	8.3	5.2	0.98
625	8.7	4.9	0.93
725	6.2	3.7	0.69
825	5.9	3.9	0.75
925	8.2	4.5	0.84
1025	7.9	3.5	0.66
1125	9.1	3.3	0.62
1225	9.2	3.5	0.65
1325	8.9	4.6	0.86
1425	7.5	3.5	0.66
1525	6.1	3.7	0.70
1625	7.0	3.0	0.57
1725	6.8	3.4	0.65
1825	7.5	3.4	0.64
1925	7.2	3.1	0.59
2025	8.6	3.6	0.67
2125	7.9	4.1	0.77
2225	7.5	4.1	0.79
2325	7.3	3.8	0.73
2425	6.2	2.7	0.54
2525	6.6	3.3	0.69
2625	6.7	2.3	0.51
2725	6.3	3.5	0.85
2825	4.4	2.3	0.65
2925	5.4	2.4	0.84
3025	7.3	4.8	1.8
3125	7.6	3.2	1.4
3225	3.0	6.6	4.6

the layers I and II—III) is the most distinct since the dividing line between the perikaryon-poor and perikaryon-rich layers is very sharp. The claustrum is also fairly sharply demarcated from the white substance. The most difficult boundary to define was that between layer VI—VII and the claustrum. Table 1 shows the depths of the borderlines of the four layers mentioned above (means, standard deviations and standard errors) obtained from above mentioned measurements. According to Table 1 the boundary between the true insular cortex and the claustral layer lies at the depth of 2000 ± 47.5 μ. The depth of the inner borderline of the claustro-insular area in this material is 2740 ± 66.5 μ.

Table 2 shows the cell density at the different depths (means, standard deviations and standard errors) measured on the same 28 histological preparations. The cell density concept in this case refers to the number of perikaryon projections larger than 80 μ^2 per *unit zone* ($100 \times 200 \times 40$ μ).

The diagram in Fig. 3 shows the number of cells per unit zone (vertical axis) at different depths (horizontal axis; zero point represented by a depth of 25 μ; see above). The means are calculated from 28 preparations. As seen in the diagram the surface layer does not include any cell projection larger than 80 μ^2 (*c.f.* layer I in Fig. 2). There are four peaks which are localised around the depths of 625 μ (*i.e.* the zone 525—625 μ), 1225 μ, 2025 μ and 3025 μ, which peaks obviously correspond to the above-mentioned horizontal perikaryon layers: II—III, V b, VI—VII and claustrum (see Fig. 2).

Table 3 shows the statistical significance of the four peaks in Fig. 3. The statistical estimation was made in such way that in each peak the zone including the largest mean cell number was compared with the intervening zones representing the smallest mean cell number. An evaluation of the significance of the differences between the means of these two types of zones was made. Table 3 shows that all four peaks are statistically significant.

Large cells. Since the positive spike potentials of large amplitude may be regarded as sings of activity from large perikarya, the largest perikarya of the population in the histological material were separated for special analysis. Thus, all perikaryon projections

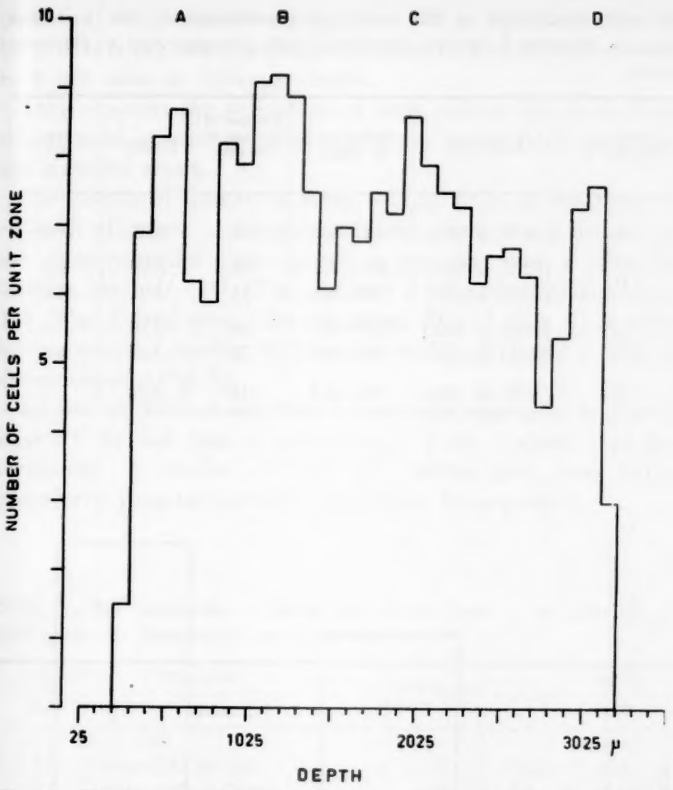

Fig. 3. Number of cells per unit zone (vertical axis) at different depths (horizontal axis). Zero point is represented by a depth of 25 μ. A, B, C and D represent main peaks.

with an area of more than 200 μ^2 were specially treated. These cells were relatively few in number and were therefore counted in four 800 μ zones which were obtained by combining 100 μ zones in four groups. Thus, the cell density in this special case is represented by the number of perikaryon projections larger than 200 μ^2 per 8 unit zones ($800 \times 200 \times 40$ μ).

The diagram in Fig. 4, shows, at different depths, the pro-

Table 3. Evaluation of the statistical significance of the principal peaks in diagram 3 showing number of cells per unit zone at different depths.

Peak in diagram 3	Zones (μ)	Difference of means	Number of degrees of freedom f	t value	Stat. sign.
A	625 & 825	8.7—5.9	54	2.37	s.
B	1225 & 825	9.2—5.9	54	3.38	h.s.
	1225 & 1525	9.2—6.1	54	3.21	h.s.
C	2025 & 1525	8.6—6.1	54	2.59	s.
	2025 & 2425	8.6—6.2	52	2.81	h.s.
D	3125 & 2825	7.6—4.4	15	2.34	s.

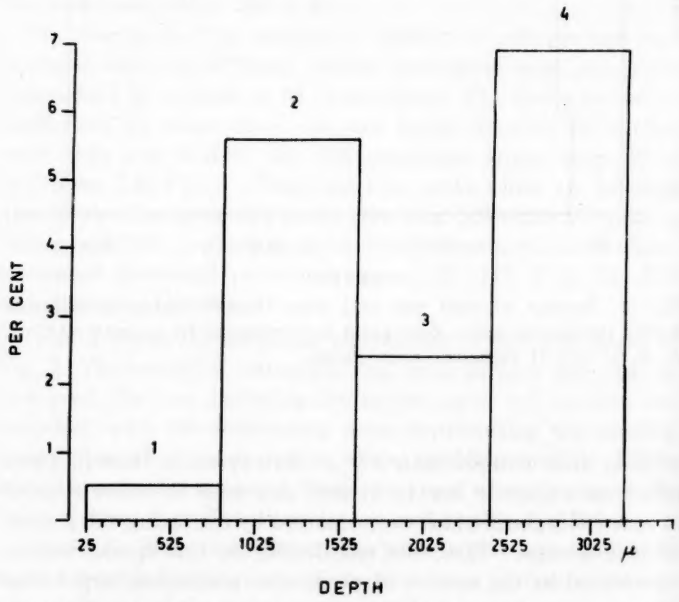

Fig. 4. Number of large cells in per cent of all cells counted in 8 unit zones (vertical axis) at different depths (horizontal axis).

portion of cell projections larger than 200 μ. This proportion of large cells is expressed in terms of the per cent of all cells counted in 8 unit zones at different depths.

Table 4 shows the proportion of large cells in per cent of all cells counted in zones 800 μ thick, and also the standard deviations and standard errors.

The calculated density of large cells in zones of this type — although arbitrary — illustrates the variations of cell density in the claustro-insular area in such a way that zone 1 (Fig. 4) includes layer II—III (Fig. 2), zone 2 (Fig. 4) includes layer V b (Fig. 2) and zone 4 the claustrum. Zone 3 (Fig. 4) is more heterogeneous, including both the layers VI—VII and a part of the claustrum (Fig. 2).

As seen in Table 4 and Fig. 4 large cells aggregate in zone 2 (layer V b) and zone 4 (claustrum). Table 5 shows that the percentage of number of large cells within these zones differ statistically from the corresponding figure for segment 3.

Table 4. The proportion of large cells in per cent of all cells per 8 unit zones. 28 histological preparations.

8 unit zones		Number of large cells	Per cent	St. E $s\{p\}$
No.	Zones (μ)			
1	from 125 to 825	5	0.50	0.21
2	from 925 to 1625	89	5.6	0.58
3	from 1725 to 2425	39	2.4	0.38
4	from 2525 to 3225	40	6.9	1.05

Table 5. Evaluation of the statistical significance of the differences between layers 4 and 3 between layers 2 and 3 as regards the relative number of large cells.

Layers (8 unit zones)	Difference of percentages	Number of degrees of freedom	t value	Stat. sign.
4 & 3	6.9—2.4	2206	2.90	h.s.
2 & 3	5.6—2.4	3214	4.64	v.h.s.

B. Distribution in depth of integrated slow activity

When the animal was in a state of deep anesthesia (DA group, see above) the slow activity varied considerably in frequency and amplitude. Sometimes the slow waves occurred in fusiform series resembling Nembutal spindles recorded from the cortical surface. Slow activity of irregular type was, however, more common. The average duration of the single waves was 50—150 msec and the amplitude 0.5 — 1.5 mV. Sometimes oscillations with higher frequency were superimposed upon the crests of the waves. Slight mechanical movements of the brain may cause a distortion in the records and changes in the slow activity due to the shift of the electrode tip was indicated by the integrator. Records in which it could be shown that oscillations were due to mechanical vibrations were omitted.

The spontaneous activity obtained could be divided into the following components:

1) Inherent noise, caused by thermal noise at the electrode tip having an amplitude of 100—200 μV. The variations of this noise were small because of the small variations in electrode impedance.

2) Background noise, consisting of irregular high-frequency low amplitude activity which increased in a typical way when the electrode approached the active cell. In several cases this buzzing could be shown to decrease when the electrode — according to the micrometer value — entered the white matter. However, since this change in activity was not always distinct it was not used systematically for the estimation of the total depth of the claustro-insular cortex.

3) Spike potentials.

4) Large slow waves.

The inherent noise and the background noise were relatively constant and are included in the integration value which constitutes the basic level. It was also found that the appearance of spike potentials had very little effect on the integration value.

Thus, the large amplitude slow waves play the dominant role in determination of the integration value, and the varying integration

values obtained at different stages of anesthesia were regarded to be due to the variations in this component.

In the majority of cases variations in the integration values were found to be due to variations in both amplitude and duration of the slow waves. As the integrator recorded activity of both polarities, the phase reversal often reported in studies of evoked responses did not appear in the results. When recordings were made during several 15 sec. periods successively with the same electrode position, the size of the integration value generally decreased with time.

In light anesthesia the slow activity was scanty and the sporadic slow waves of large size did not contribute appreciably to the integration values. In aging preparations variable and irregular slow waves with a duration of 300—500 msec. were recorded. In these cases there was no spatial differentiation of the slow activity.

Some typical examples will be presented in order to illustrate the distribution of the integrated activity in different cortical layers recorded at successive electrode insertions. The influence of the depth of anesthesia on the integrated activity in different cortical layers will also be described.

Fig. 5 shows the relative values for the integrated activity at different cortical depths obtained from three electrode insertions (I_1, I_2 and I_3). The electrode was moved in 10 μ steps down to a depth of 500 μ. The electrode impedance was constant.

Curve I_1 illustrates the situation in deep anesthesia. Curve I_2 was obtained in a later phase, when an additional dose of Nembutal had been injected and curve I_3 shows the spatial distribution of the slow activity some hours later. Judged by the narcotic level of the preparation, the values obtained in the first two series (I_1 and I_2) belong to the DA group (see above) and the values of the third series (I_3) to the LA group. The curve based on the integration values from the first insertion (I_1) show certain irregularities. This type of curve was often obtained in the initial phase of Nembutal anesthesia during which the slow activity is labile, as was mentioned above. The second insertion was made after an additional dose of Nembutal which was followed by an increase of the slow activity. The curve (I_2 in Fig. 5) showing the spatial distribution of the integrated activity obtained at the

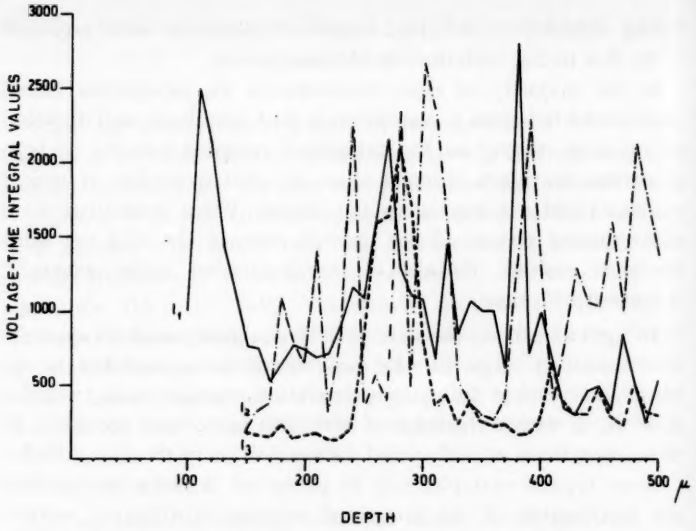

Fig. 5. Relative voltage-time integral values at different depths obtained from three successive electrode insertions (I_1 and I_2 belonging to the DA group and I_3 to the LA group). The electrode was moved in 10 μ steps down to a depth of 500 μ.

second insertion shows a *maximal* peak at a depth of 300 μ. This maximum is of special interest since it was found to be most typical for experiments of this type. As seen in Table 1 the deep boundary of the first cortical layer is to be found at a depth of 320 ± 8.2 μ. The highest peak having its maximum at 300 μ thus seems to correspond to the zonal lamina or the boundary between this layer and the first pyramidal cell layers proper (II and III in Table 1 and Fig. 2). There is a corresponding dominating peak in the curve based on the values from the third insertion (I_3), the values of which indicate a diminished slow activity.

The curves in Fig. 6 based on values from two successive electrode insertions in another rabbit show the same general characteristics. The values represented by curve I_1 were obtained in a

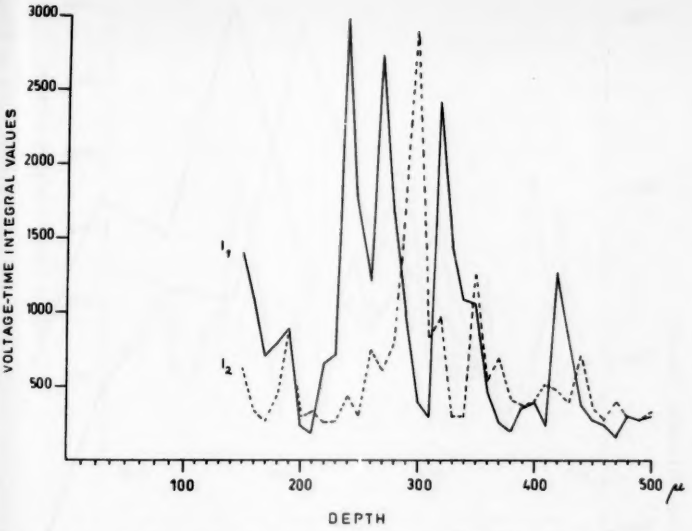

Fig. 6. Relative voltage-time integral values at different depths obtained from two successive electrode insertions (I_1 belonging to the DA group and I_2 to LA group). The electrode was moved in 10 μ steps down to a depth of 500 μ.

deep stage of anesthesia (DA) and the maximum of the curve corresponds to a depth of about 250 μ. Curve I_2 in Fig. 6 is based on values from the second insertion which was made in a stage of light anesthesia (LA). As seen the maximum of the curve corresponds to a depth of 300 μ.

In Fig. 7 the curves are based on values from three successive insertions in the claustrum, the two first insertions (I_1 and I_2) being made in deep anesthesia (DA) and the third (I_3) in light anesthesia (LA). In this experiment the electrode was moved in 100 μ steps. The curves based on the values from the first two insertions made in deep anesthesia are similar and their peak values are found at a depth of 2800—2900 μ, which depth corresponds to the deepest part of the claustrum. As was the case in the experiments described above the slow activity was reduced

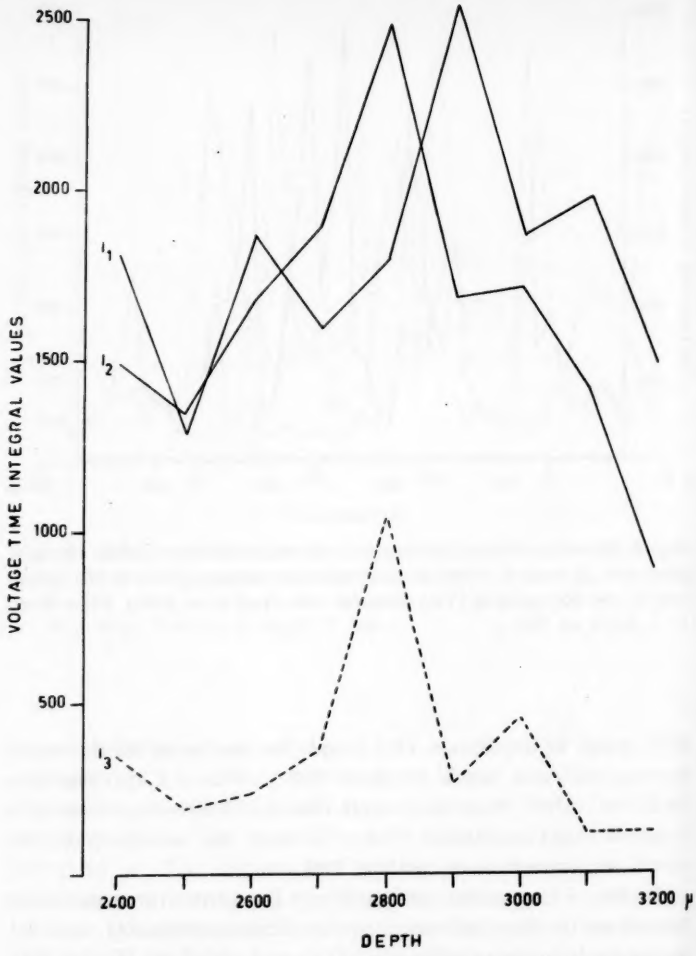

Fig. 7. Relative voltage-time integral values at different depths between 2400 and 3200 μ obtained from three successive electrode insertions I_1 and I_2 belonging to the DA group and I_3 to the LA group). The electrode was moved in 100 μ steps.

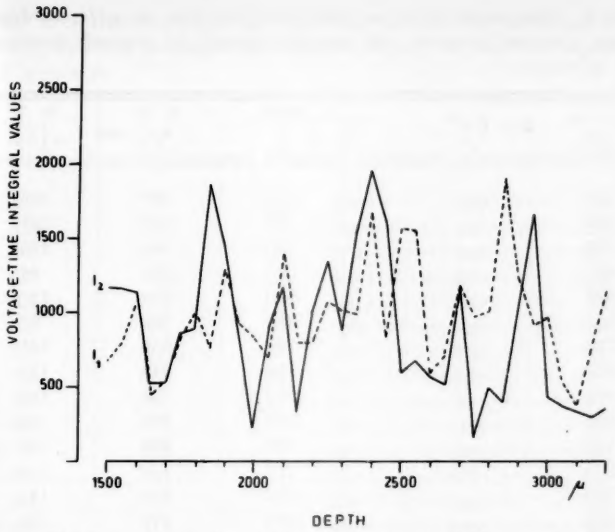

Fig. 8. Relative voltage-time integral values at different depths between 1500 μ and 3200 μ obtained in an experiment in which two successive insertions were made in light anesthesia (LA group). Curve I_1 shows the values obtained from the first insertion which was made in 50 μ steps and curve I_2 shows the values obtained at every 50 μ step from the second insertion, which was made in 10 μ steps.

in light anesthesia as indicated by curve I_3 the peak of which showed the same position as curve I_1 and I_2 around the depth of 2800—2900 μ.

Fig. 8 illustrates the integrated activity at varying depths between 1500 and 3200 μ obtained in an experiment in which two successive insertions were made in light anaesthesia. Curve I_1 shows the values obtained in the first insertion which was made in 50 μ steps and curve I_2 the values obtained at every 50 μ step from the second insertion which was made in 10 μ steps. Insertion 1 (curve I_1) took 30 minutes and insertion 2 (curve I_2) 90 minutes. The experiment shows that the curves obtained on the basis of values from two insertions made at the same anesthetic level but with different time course have similar general shape.

Table 6. Integration value per unit zone (see text) at different depths (mean, standard deviation and standard error). 14 electrode insertions. DA group.

Zone (μ)	Mean $\bar{x}$	St. D s_x	St. E $s_{(\bar{x})}$
125	900	590	164
225	830	630	167
325	580	390	105
425	500	240	64
525	590	450	120
625	510	350	93
725	760	530	141
825	750	640	171
925	650	390	105
1025	630	280	74
1125	680	460	122
1225	730	440	119
1325	630	440	118
1425	650	470	126
1525	650	490	131
1625	580	270	73
1725	740	400	107
1825	760	330	102
1925	880	680	183
2025	650	370	98
2125	570	320	86
2225	690	380	102
2325	670	690	184
2425	780	460	122
2525	770	760	203
2625	580	520	139
2725	740	610	170
2825	740	470	135
2925	990	850	257
3025	790	820	248
3125	800	760	269
3225	600	420	148

The material treated statistically comprised data from 14 electrode insertions (9 animals) from the DA group and 17 (9 animals) from the LA group. Recording data from a maximum of three electrode insertions per animal was included in the material.

Table 7. Integration value per unit zone (see text) at different depths (mean, standard deviation and standard error). 17 electrode insertions. LA group.

Zone (μ)	Mean $\bar{x}$	St. D s_x	St. E $s\{\bar{x}\}$
125	460	330	82
225	700	650	158
325	570	790	191
425	480	430	104
525	400	250	59
625	560	520	126
725	480	370	91
825	440	290	70
925	490	380	92
1025	430	260	63
1125	450	430	105
1225	400	270	66
1325	470	340	82
1425	380	180	45
1525	380	370	90
1625	360	220	54
1725	390	290	70
1825	370	270	66
1925	430	330	81
2025	440	380	93
2125	360	300	72
2225	350	210	51
2325	340	262	64
2425	410	330	80
2525	400	480	116
2625	400	390	98
2725	440	400	98
2825	510	430	103
2925	420	390	95
3025	420	410	100
3125	350	160	53
3225	380	350	116

Tables 6, 7 and 8 show the mean integration values and the differences of the means for both groups. Fig. 9 shows the distribution in depth of the mean integration values for both groups (DA group represented by full lines and LA group by dashed lines).

Table 8. Evaluation of the statistical significance of the difference between the DA and LA groups as regards the integration values per unit zones at different depths.

Zones (μ) (DA & LA)	Difference of means	Number of degrees of freedom f	t value	Stat. sign.
125	900—460	27	2.58	s.
225	830—700	29	0.56	—
325	580—570	29	0.07	—
425	500—480	29	0.21	—
525	590—400	29	1.50	—
625	560—510	29	0.30	—
725	760—480	29	1.74	—
825	750—440	29	1.76	—
925	650—490	29	1.14	—
1025	630—430	29	2.02	—
1125	680—450	29	1.40	—
1225	730—400	29	2.49	s.
1325	630—470	29	1.14	—
1425	650—380	29	2.22	s.
1525	650—380	29	1.74	—
1625	580—360	29	2.46	s.
1725	740—390	29	2.82	h.s.
1825	760—370	29	3.34	h.s.
1925	880—430	29	2.42	s.
2025	650—440	29	1.50	—
2125	570—360	29	1.89	—
2225	690—350	29	3.18	h.s.
2325	670—340	29	1.83	—
2425	780—410	29	2.60	s.
2525	770—400	29	1.64	—
2625	580—400	28	1.07	—
2725	740—440	28	1.62	—
2825	740—510	27	1.38	—
2925	990—420	28	2.38	s.
3025	790—420	26	1.59	—
3125	800—350	15	1.76	—
3225	600—380	15	1.17	—

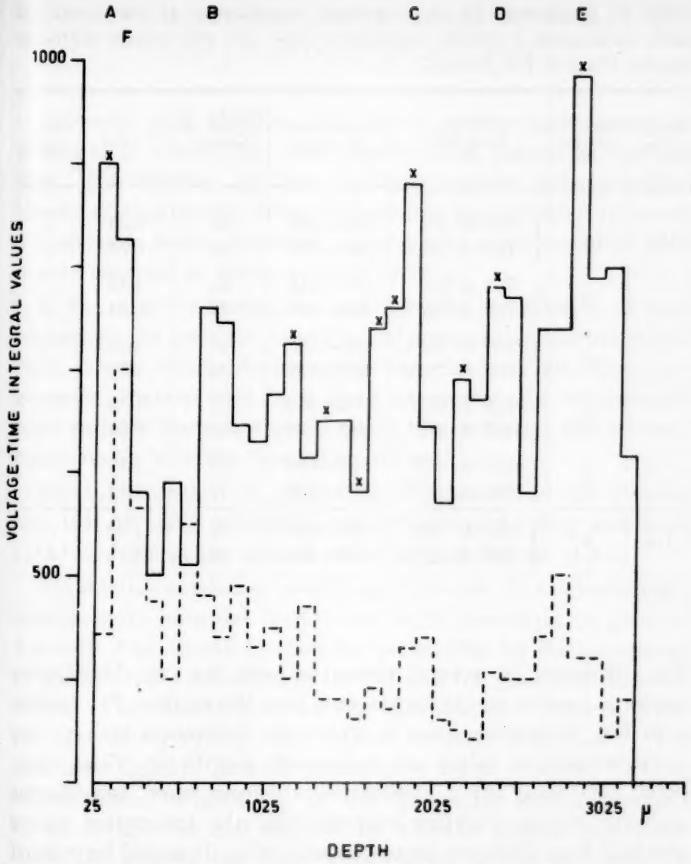

Fig. 9. Distribution in depth of the mean integration values for group DA (full line) and for group LA (dashed line). The symbol x) signifies zones in which the difference between the mean DA and LA integration values is statistically significant. A, B, C, D, E and F represent the main peaks.

Table 9. Evaluation of the statistical significance of the principal peaks in diagram 9 showing integration value per unit zone at different depths. DA and LA groups.

Anesth. group	Peak in diagram 9	Zones (μ)	Difference of means	Number of degrees of freedom f	t value	Stat. sign.
DA	A	125 & 425	900—500	25	2.35	s.
		125 & 325	900—580	26	1.75	—
	B	725 & 625	760—510	26	1.49	—
		725 & 1025	760—630	26	0.84	—
	C	1925 & 1625	880—580	26	1.52	—
		1925 & 2125	880—570	26	1.53	—
	D	2425 & 2125	780—570	26	1.36	—
		2425 & 2625	780—580	26	1.07	—
	E	2925 & 2625	990—580	23	1.47	—
		2925 & 3225	990—600	17	1.17	—
LA	F	225 & 125	700—460	31	1.32	—
		225 & 525	700—400	32	1.78	—

The difference in general course between the two distribution curves is notable except for the area near the surface. The symbol x in Fig. 9 signifies zones in which the differences between the mean integration values are statistically significant. Thus, Nembutal, like many other anesthetics, enhances slow spontaneous activity. It was therefore expected that the integration values obtained from these two groups should differ. It should be pointed out that the DA group does not represent recordings made under very heavy anesthesia in which the slow wave activity decreases.

Table 9 shows the statistical evaluation of some the most significant peaks in the integration curves. The only significant peak value was that found near the surface in the DA group corresponding to the zonal lamina. The variation of the integration values was to great to permit a further accurate analysis along this line.

C. The distribution in depth of positive spike potential activity

Although main attention was paid to positive spike potentials, it should be pointed out that negative spike potentials were also seen. The number of these initially negative spike potentials clearly distinguishable from the base line was too small to permit a systematic study. Generally, the negative spike potentials were mostly obtained at depths of 1000—2000 μ.

In the majority of cases the positive spike potentials — or more accurately the positive - negative and monophasic positive potentials — were clearly distinguished from the base line. They were generally characterized by a large amplitude and consequently were suitable for a systematic study. The material was collected concurrently with the integration values.

The total number of positive spike potentials in the material was 164; 59 being obtained in the animals under deep anesthesia (DA) and 105 in the animals under light anesthesia (LA).

The relative number of recordings (duration 15 sec) including a positive spike potential (in per cent of all recordings) is given in Table 10. Fig. 10 and 11 show the probability for the appearance of positive spike potentials in the DA and LA groups at different depths. The distribution curves resemble each other in their general configuration but display certain differences. The maximum appears in both groups at a depth of about 1000 μ., corresponding to layer V_b. In both groups the peak is preceded by an abrupt rise and followed by a gentle general fall in the curve. In general the distribution curves of the DA and LA groups are almost identical from the surface to a depth of about 1030 μ, but from there on the curves show that in the deeper layers a greater number of positive spike potentials were obtained in light than in deep anesthesia.

The distribution curves of both groups show local "accumulations" of spike activity, the statistical significance of which is shown in Table 11. According to this table, the only statistically significant accumulation in the DA group was localized at a depth of around 1000 μ *i.e.* in the layer of the large pyramidal

Table 10. Number of 15 sec. recordings including a positive spike potential per unit zone at different depths and number of positive spike recordings in per cent of all recordings at different depths. DA group (14 electrode insertions) and LA group (17 electrode insertions).

Zone (μ)	DA group			LA group		
	Number of positive spike recordings	Per cent	St. E $s\left\{p\right\}$	Number of positive spike recordings	Per cent	St. E $s\left\{p\right\}$
125	—	—	—	—	—	—
225	—	—	—	—	—	—
325	2	7.2	4.9	2	5.9	4.0
425	1	3.6	3.5	4	11.8	5.5
525	4	14.3	6.6	3	8.8	4.9
625	3	10.7	5.9	3	8.8	4.9
725	3	10.7	5.9	3	8.8	4.9
825	1	3.6	3.5	5	14.7	6.1
925	4	14.3	6.6	2	5.9	4.0
1025	6	21.4	7.8	5	14.7	6.1
1125	5	17.9	7.3	3	8.8	4.9
1225	3	10.7	5.9	8	23.6	7.3
1325	1	3.6	3.5	2	5.9	4.0
1425	3	10.7	5.9	2	5.9	4.0
1525	2	7.2	4.9	7	20.6	6.9
1625	1	3.6	3.5	7	20.6	6.9
1725	3	10.7	5.9	2	5.9	4.0
1825	2	7.2	4.9	2	5.9	4.0
1925	2	7.2	4.9	4	11.8	5.5
2025	—	—	—	2	5.9	4.0
2125	3	10.7	5.9	5	14.7	6.0
2225	1	3.6	3.5	7	20.6	6.9
2325	1	3.6	3.5	3	8.8	4.9
2425	1	3.6	3.5	2	5.9	4.0
2525	1	3.6	3.5	3	8.8	4.9
2625	1	3.6	3.5	5	15.7	6.4
2725	2	7.7	5.2	4	11.8	5.5
2825	—	—	—	3	8.8	4.9
2925	1	4.6	4.4	3	8.8	4.9
3025	—	—	—	1	2.9	2.9
3125	1	6.3	6.1	3	16.7	8.8
3225	1	6.3	6.1	—	—	—

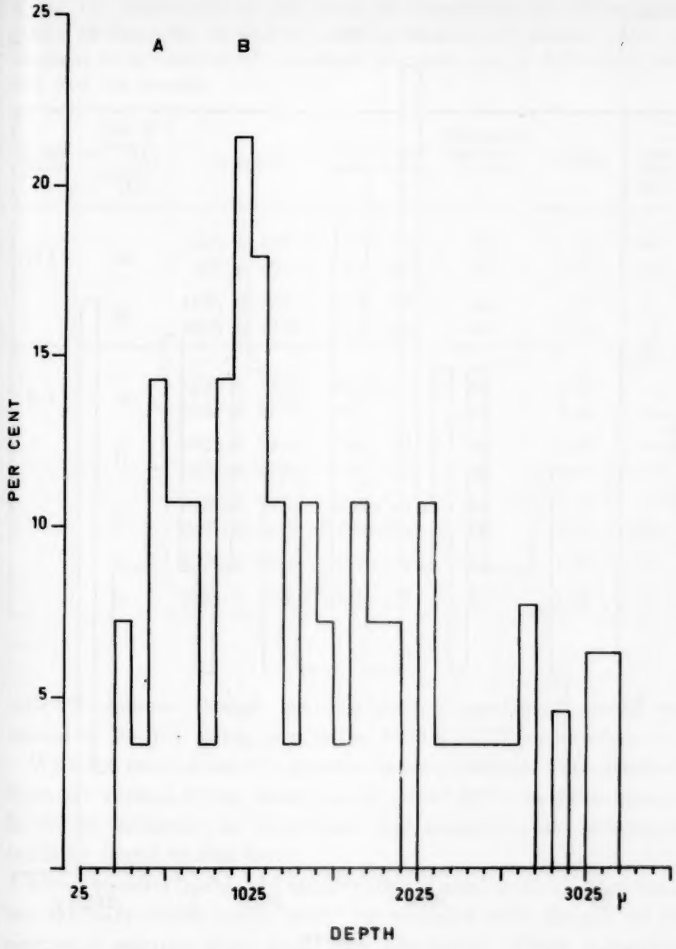

Fig. 10. Probability of appearance of positive spike potentials in the *DA group* at different depths. The probability expressed as per cent of 15 sec. recordings that showed a positive spike potential.

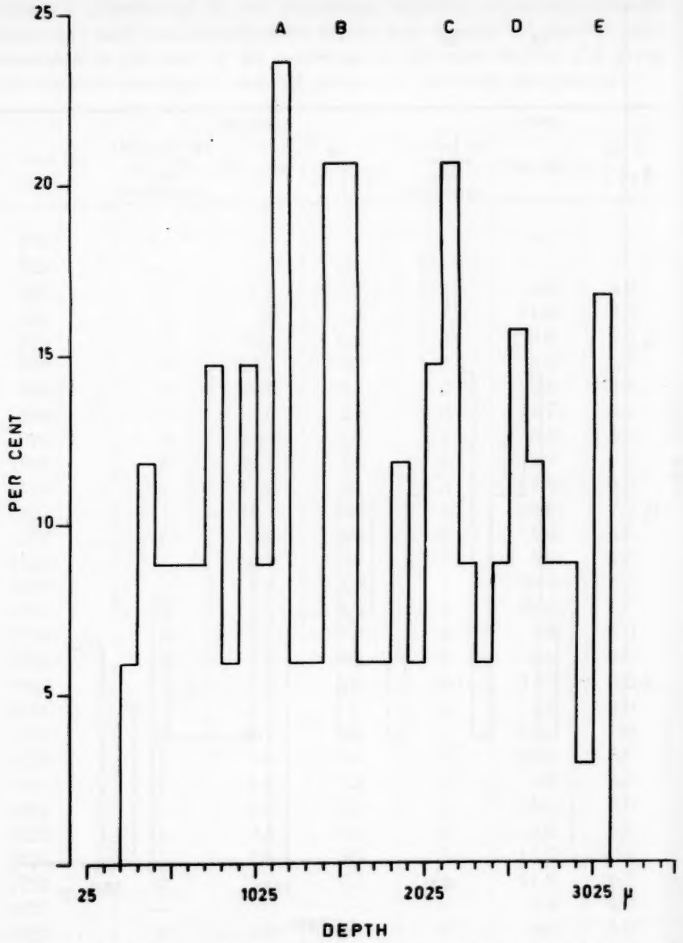

Fig. 11. Probability of appearance of positive spike potentials in the *LA group* at different depths.

Table 11. Evaluation of the statistical significance of the principal peaks in diagrams 10 and 11 showing number of positive spike recordings in per cent of all recordings per unit zone at different depths. DA and LA groups.

Anesth. group.	Peak in diagram 10 (DA) respect. 11 (LA)	Zones (μ)	Difference of percentages	Number of degrees of freedom f	t value	Stat. sign.
DA	A	525 & 425	14.3—3.6	54	1.43	—
		525 & 625	14.3—10.7	54	0.41	—
	B	1025 & 825	21.4—3.6	54	2.10	s.
		1025 & 1325	21.4—3.6	54	2.10	s.
LA	A	1225 & 1125	23.6—8.8	66	1.69	—
		1225 & 1325	10.7—3.6	66	2.12	s.
	B	1525 & 1425	20.6—5.9	66	1.83	—
		1525 & 1725	20.6—5.9	66	1.83	—
	C	2225 & 2025	20.6—5.9	66	1.83	—
		2225 & 2425	20.6—5.9	66	1.83	—
	D	2625 & 2425	15.7—5.9	64	1.30	—
	E	3125 & 3025	16.7—2.9	50	1.49	—

cells. Prominent though not statistically significant peaks occurred in the LA group also at the 1500 and 2200 μ depths.

With the method used no positive spike potentials were obtained from the surface layers down to a depth of 200 μ in either group. It should, however, be mentioned that negative spike potentials could be found in this layer.

Large positive spike potentials with an amplitude of more than one millivolt which could easily be recorded with the aid of an electronic counter were dealt with separately. These potentials were also either positive-negative or monophasic positive. The duration of the negative deflections varied and when the duration of the negative phase was at it greatest the configuration of the potential was as shown in Fig. 12. The average frequency of such potentials in the DA group was 1—2 per sec and in the LA group 4—5 per sec. Since recordings which displayed positive spike

Fig. 12. Configuration of an initial positive diphasic spike potential. Calibration: Time bar 10 msec. Voltage bar 0.5 mV.

potentials with an amplitude over one millivolt were relatively few (20 in the DA group and 30 in the LA group) the 8 unit zones were used with the total cortical depth being divided into four zones: 25—825 μ, 825—1625 μ, 1625—2425 μ and 2425—3225 μ. Fig. 13 shows the probability for the appearance of such potentials in these different zones obtained from all recordings (15 sec duration). The diagram indicates that the probability for the appearance of the large positive potentials at different depths is different in the two groups. In deep anesthesia these potentials appear most frequently in zone 2 whereas in light anesthesia they appear most frequently in zone 4.

The number of recordings with a large positive spike potential in per cent of all recordings made from the different zones in both groups are given in Table 12. Table 13 shows the statistical significance of the difference between the groups as regards the probability of the appearance of the large positive spike potentials in zones 1 and 4. Fig. 13 shows that these large positive spike potentials tended to appear more often in the zones 2 and 4 than in the intervening ones. The similarity between the diagram in Fig. 13 with that in Fig. 4 showing the distribution of large cells at different depths indicates that the main origins of the large spike potentials are situated in layers containing the largest perikaryon. The difference between the LA and DA group in zone 4

Table 12. The proportion of large positive spike recordings in per cent of all recordings per 8 unit zones. DA and LA groups.

Anesth. group	8 unit zones		Number of large positive spike recordings	Per cent	St. E $s\{p\}$
	No.	Zones (μ)			
DA	1	from 125 to 825	6	2.71	1.08
	2	from 925 to 1625	9	4.02	1.31
	3	from 1725 to 2425	4	1.79	1.33
	4	from 2525 to 3225	1	0.55	0.55
LA	1	from 125 to 825	1	0.37	0.37
	2	from 925 to 1625	11	4.05	1.20
	3	from 1725 to 2425	8	2.94	1.03
	4	from 2525 to 3225	10	4.20	1.30

Table 13. Evaluation of the statistical significance of the differences between layers 1_{DA} and 1_{LA} and between layers 4_{DA} and 4_{LA} as regards the percentages of large positive spike recordings.

Layers (8 unit zones)	Difference of percentages	Number of degrees of freedom f	t value	Stat. sign.
1_{DA} & 1_{LA}	2.71—0.37	490	2.04	s.
4_{DA} & 4_{LA}	4.20—0.55	418	2.59	h.s.

shown by Fig. 13 is of special interest. According to Table 13 this difference is highly significant. Fig. 13 thus shows that the large claustral cells exhibit a pronounced spontaneous activity of this type in light anesthesia as compared with the state of deep anesthesia. No such difference is found in zone 2 corresponding to layer V. On the contrary the spontaneous activity in this layer is of the same intensity in light and deep anesthesia. Thus, the large insular pyramidal cells in layer V c do not react to changes in anaesthesia as critically as the large claustral cells.

The spatial extent of the proximity potentials is regarded not to be much larger than the diameter of the soma which serves as

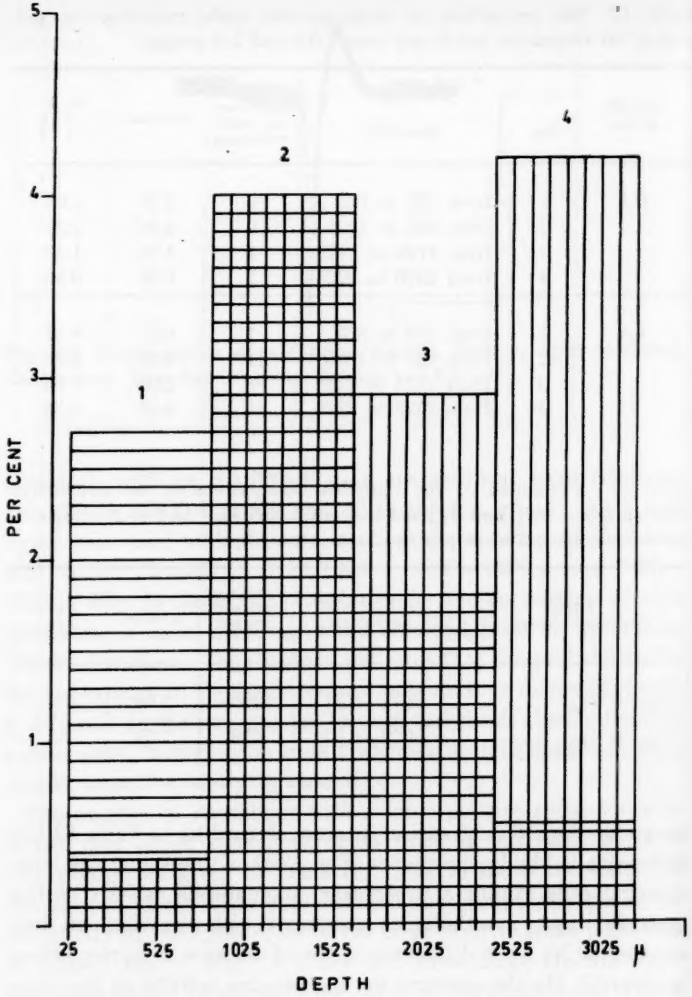

Fig. 13. Probability of appearance of large positive spike potentials in different layers (8 unit zones). DA and LA group.

origin (Li and Jasper 1953). Therefore they may not be found when the electrode is moved in 50 μ steps. They are more likely to be observed when the distances between the recording points are only 10 μ. Using such a procedure would, however, take too long time for a comparison of the results obtained near the surface with those obtained from the depth (see above). Such an examination was therefore limited to some few layers only. It was possible to record the same spike potential in the majority of cases at three successive recording points, *i.e.* over a distance of 30 μ. The greatest recording distance within which the same potential could be recorded was found to be 70 μ, within the claustral area.

D. Correlation between the density of claustro-insular cells and the electrophysiological data

Table 14 in which the morphological and functional data are presented shows that there is a highly significant positive correlation between the probability for the appearance of positive spike potentials and the density of cells in the DA group. The table also shows that there is a statistically significant negative correlation between the integrated slow activity and the density of cells in the DA group. In the LA group on the other hand the correlation between the electro-physiological data and the density of cells is not statistically significant.

Table 14. Correlations between the density of claustro-insular cells and the electrophysiological data. (PSP.a. = positive spike potential activity; INT.a. = integrated activity.) In all cases the number of degrees of freedom = 28.

Factors participating in the correlation	Correlation coefficient r	t value	Stat. sign.
Cells/PSP.a.$_{DA}$	+ 0.51	3.28	h.s.
Cells/INT.a.$_{DA}$	— 0.37	2.18	s.
Cells/PSP.a.$_{LA}$	+ 0.26	1.43	—
Cells/INT.a.$_{LA}$	— 0.20	1.14	—

E. Correlation between integrated activity and positive spike potential activity

From what has been said above a negative correlation between the positive spike potentials and the integrated slow activity is to be expected in the DA group. Table 15 shows that there is actually a statistical significance of this negative correlation both in the LA and DA group. If, on the other hand, the correlations in the two groups are examined crosswise, as shown in Table 15, the statistical value of the correlation coefficient is not significant.

The data obtained above can examined also by means of partial correlation (see Methods). If positive spike potential activity and integrated activity are correlated under the assumption that the cell density remains constant throughout whole cortical depth, the value of the coefficient (PSP.a/INT. a) changes in group DA to —0.23 (difference 0.14) and loses its statistical significance. In the LA group this change is smaller and the value of the coefficient becomes —0.37 (difference 0.03), which is still significant.

The weight of the negative correlation must be assessed from the standpoint of the method used. Integrated activity and spike potential activity were studied simultaneously during periods with a duration of 15 seconds. As has been pointed out the potential pattern remains the same at a certain point during the time for recording and it has also been found that at certain

Table 15. Correlations between integrated activity (INT.a.) and positive spike potential activity (PSP.a.). In all cases number of degrees of freedom = 28.

Factors participating in the correlation	Correlation coefficient r	t value	Stat. sign.
$PSP.a._{DA}/INT.a._{DA}$	— 0.37	2.18	s.
$PSP.a._{LA}/INT.a._{LA}$	— 0.40	2.41	s.
$PSP.a._{DA}/INT.a._{LA}$	— 0.09	0.49	—
$PSP.a._{LA}/INT.a._{DA}$	— 0.22	1.24	—

depths solely slow activity may occur whereas positive spike potentials and less slow activity is characteristic for intervening layers. However, in some experiments pronounced slow activity and positive spike potentials appeared during the same 15 sec period. In these cases, however, the electrode position was found to be critical. A small change in the position was enough to cause a complete change in pattern.

The above correlations are illustrated graphically in Fig. 14 which gives the values from one electrode insertion made with 10 μ steps. The insertion is the same as in Fig. 8 (insertion 1). Every integration value obtained is plotted and the positive spike potentials observed are marked. The diagram illustrates data obtained from depths between 2000—3000 μ, corresponding to the claustrum. Fig. 14 shows that positive spike potentials occur at depths between 2490—2640 μ and between 2980—3080 μ and that the integration values are reduced at the corresponding depths. At a depth of 2000—2300 μ however, the integration values are low although no positive spike potentials are recorded simultaneously.

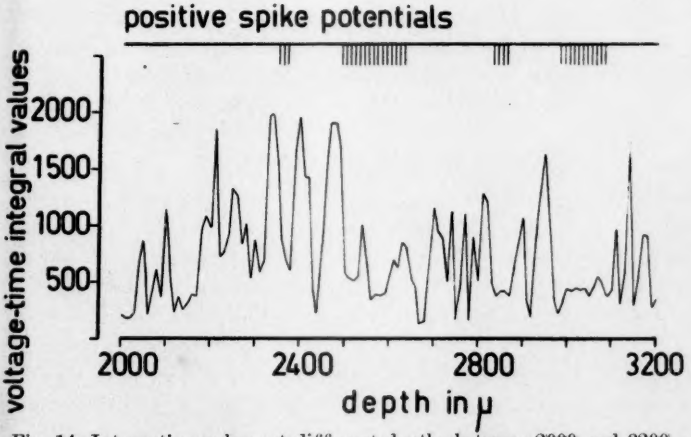

Fig. 14. Integration values at different depths between 2000 and 3200 μ obtained from one electrode insertion. The electrode was moved in 10 μ steps. The insertion is the same as in Fig. 8 (I_2). The depths at which positive spike potentials were obtained are indicated by arrows.

VI. DISCUSSION

The present study of the different types of electrical activity in the various cortical layers is based on histological studies of nerve cell density and electrophysiological data obtained from experiments in which the depth of the microelectrode tip was estimated from a micrometer scale on the micromanipulator. When comparing such histological and functional data, one must consider errors which may be introduced by structural changes in the histological preparations and by difficulties in the estimation of the exact position of the electrode tip (*e.g.* dimpling of the cortical surface, non-perpendicular direction of the electrode insertion etc.). However, it is known that the positive spike potentials are readily obtained from layers rich in nerve cells and in the present material a highly significant correlation was found between the probability of the appearance of positive spike potentials and cell density at different depths. Therefore it can be assumed that the measurements of the depths of the electrode tip are equivalent to the depth measurements obtained from the histological preparations.

The statistical treatment of the results obtained from the animals under deep anesthesia shows a negative correlation between the integrated slow activity and the positive spike potential activity, the latter being obtained from the perikaryon rich layers. Before discussing this negative correlation the significance of the positive spike potentials should be considered.

In these investigations the electrode was always moved in fixed steps rather than continuously in order to enable the studies of the correlation of the functional and structural data to be based on a material which could be treated statistically. Under these experimental conditions it is not known to what extent the recorded positive spike potential may be considered

spontaneous and not as the result of mechanical stimulation of the cell by the electrode tip. It is obvious that for a frequency study this factor would be of importance. However, the aim of these investigations has not been to study the frequency characteristics of the cortical cells, and the conclusions are not based on any data concerning discharge frequency.

The typical injury potentials which appear in trains with a high accelerating frequency have always been exluded from this material. However, it has been suggested that the appearance of initial positive spikes alone would indicate that the cell from which that spike activity is led off is damaged. Thus, MOUNTCASTLE *et al.* stated that the absence of slow background activity tends to be associated with initially positive spike potentials: "The appearance of such a discharge is always accompanied by an increased degree of isolation *i.e.* by a marked decrease in the background neuronal activity recorded". (MOUNTCASTLE *et al.* 1957). This could mean that when initially positive spike potential activity is recorded, the conditions are unfavourable to obtain slow activity due to damage of the cells in the vicinity of the electrode tip. One could thus argue that the negative correlation between integrated slow activity and positive spike potential activity may be explained on this basis.

On the other hand it has been shown that the positive polarity of a recorded spike potential does not necessarily indicate cell injury if the frequency of the spike potentials does not rise to the so called injury level. Several workers have reported that the same positive spike potential can be recorded at a certain depth even if the electrode is moved in the vicinity of the cell (GRANIT & PHILLIPS 1956; MOUNTCASTLE *et al.* 1957). MOUNTCASTLE and co-workers (1957) have also suggested the possibility that such an initially positive spike potential represents capacitive recording with the tip of the electrode pressing against the cell membrane. The appearance of isolated initially positive spike potentials without any background activity may also indicate that the electrode tip has attained a critical position between a region characterized by giving a graded slow potential and a region characterized by giving an all-or-none type spike potential (see CLARE & BISHOP 1955 b).

The negative correlation between the integrated slow activity and the positive spike potentials would thus mean that in deep anesthesia the slow activity is less pronounced in the perikaryon rich layers than in the intervening layers characterized by a low cell density. Thus, there ought to be a negative correlation between the integrated slow activity and the cell density in deep anesthesia. Although the results of the individual experiments presented in chapter V b suggest that this is the case, statistical significance could not be obtained except for the superficial layer. The lack of statistical significance may be due to several factors. The recordings were not made simultaneously from all the different points at varying depths and the slow activity at different levels may change somewhat in the course of the experiment. The relatively short recording time may be another reason for the statistical variations. A recording time of 15 seconds was chosen as the result of a compromise. A prolongation of the recording time would have lengthened critically the time for the total experiment and thus impaired the comparability of the results obtained from the surface and from the deeper structures. DROCHOCKI (1956) studied the integrated EEG activity in a cortical area and observed that the variations disappeared almost completely in successive recordings when the recording time was lengthened to 200—300 sec. Finally, the integrated slow activity may be heterogeneous and the relation between its different components may change with cortical depth.

An increase in activity of short duration was frequently observed after the electrode had been moved to a new position. This increase of activity was assumed to be due to mechanical stimulation caused by the electrode tip and possibly also due to the injury of some units. Therefore, integration was not started until 5 seconds after moving the electrode, during which interval the activity reached a constant level.

Variations in the depth of anesthesia within the range tested had no significant effect on the negative correlation between positive spike potential activity and integrated slow activity. However, the positive correlation between spike potential activity and cell density is significant in deep anesthesia only. It follows that the negative correlation between integrated slow activity and cell density is significant in deep anesthesia only.

This difference in the distribution of spike activity and slow activity which seems to exist between light and deep anesthesia needs not concern the superficial and deep cortical layers uniformly. The large insular pyramidal cells in layer V c do not react to changes in anesthesia as critically as the large claustral cells, as was mentioned on page 45. The last mentioned observation points to a differential behaviour of these two neuron structures which are both characterized by a high density of large nerve cells.

According to DEMPSEY and MORRISON (1942 a, b) the typical spindle activity in animals under barbiturate anesthesia follows the same functional laws as the recruiting phenomenon. This phenomenon is dependent on activation mediated by the so-called unspecific thalamo-cortical pathways. According to LORENTE DE NÓ (1951), the distribution of the unspecific thalamo-cortical afferents is different from that of specific afferents. He claimed that the net-work of unspecific fibres terminates in the cortex diffusely in contrast to the relatively precise localization of the specific thalamo-cortical pathways. Electrophysiological results pointing in the same direction have been obtained in microelectrode studies by LI *et al.* (1956). They suggest the possibility that dendritic depolarization may occur in response to unspecific afferent volleys, even without discharge of the cortical cells.

It may thus be that the axo-dendritic connections of the unspecific pathways in the claustro-insular area influence the distribution in depth of integrated slow activity.

VII. SUMMARY

1. In the present investigation the distribution of the spike and slow wave activities was studied with an extracellular microelectrode technique in different layers throughout the entire cortical depth. The claustro-insular area in the rabbit was chosen as the object for this study and a description of this area *(area insularis agranularis anterior dorsalis)* is presented in Chapter III.

2. The spike and slow wave activity was led off with a KCl-filled glass capillary electrode which was inserted in stepwise fashion to different depths in the claustro-insular area. Each insertion was made in 50 μ steps perpendicular to the cortical surface, the depth of the electrode tip under the surface being estimated by the aid of a micrometer attached to the micromanipulator. The slow potentials at each electrode position were integrated electronically during fixed intervals of time, and the probability of the appearance of positive spike potentials during the same time periods was estimated (see Recording in Chapter IV).

3. Diagrams in which the time integral values for the slow wave activity and the probability of appearance of positive spike potentials were plotted against depths in μ, show the distribution in depth of these two types of cortical activity. The material was treated statistically. (Chapters V B and V C).

4. The distribution in depth of the two types of activity was studied on animals under light anesthesia (LA group; see page 15) and on animals under deep anesthesia (DA group; see page 15).

5. The density at different cortical depths of the claustro-insular cells, the projections of which exceeded 80 μ^2 (see page 18), was studied on histological preparations obtained from perpendicular

sections from 28 animals which were not used in the electrophysiological experiment (see Histological preparation in Chapter IV).

6. Diagram in which the number of cells per unit zone (see page 25) was plotted against depths in μ, illustrate the distribution of the counted nerve cells in the different cortical layers. The material was treated statistically (Chapter V A).

7. On the basis of the electrophysiological data obtained, the correlation between the appearance of the slow activity and spike potentials was studied (Chapter V E). A significant negative correlation was found between the appearance of slow activity and spike potentials both in light ($r = -0.40$) and in deep anaesthesia ($r = -0.37$).

8. On the basis of the electrophysiological and the histological material the distribution of the spike and slow wave activities was correlated with the density of claustro-insular cells.

9. A positive correlation between spike activity and cell density was found to be highly significant in the animals under deep anesthesia ($r = +0.51$). This positive correlation was non-significant in the animals under light anesthesia ($r = +0.26$).

10. A significant negative correlation was found between the slow activity and the cell density in the animals under deep anesthesia ($r = -0.37$). This negative correlation was non-significant in the animals under light anesthesia ($r = -0.20$).

11. The difference between light and deep anesthesia as regards the distribution of spike activity and slow activity seems not to concern the superficial and deep cortical layers uniformly, since it was found that the large insular pyramidal cells in layer V C did not react to changes in anesthesia as critically as the large claustral cells.

12. On the basis of the results obtained, the dendritic origin of the slow wave activity correlated to the layers characterized by a low cell density was discussed.

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